The Approach to Abnormal Liver Enzymes in Companion Animals

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Abnormal liver enzyme activity on a biochemical profile is commonly found in companion animals.[1] Abnormal liver enzymes are either identified in the clinically normal patient having a routine health or pre-anesthetic screen prior to an elective procedure or during a complete diagnostic work-up when the patient is sick. The sick patient with abnormal liver enzymes may have primary liver disease or disease secondary to non-hepatic disorders or drugs that affect liver enzyme production. The clinician is often left with questions on how to deal with these case scenarios in a logical approach. To begin, the clinician must understand why liver enzymes become abnormal prior to examination of their patients. Liver enzymes can be insensitive or nonspecific for primary liver disease and some have isoenzymes from other tissues not associated with the liver. Routine liver biochemical tests can be categorized into the following groups that reflect:

1. Hepatocellular injury (alanine aminotransferase [ALT], aspartate aminotransferase [AST])
2. Cholestasis/enzyme induction (alkaline phosphatase [ALP], gamma-glutamyl transferase [or transpeptidase; GGT])
3. Tests reflecting impaired metabolic function or synthetic capacity

Commonly, additional diagnostics may be required to reach a specific diagnosis, including bile acids, coagulation testing, imaging studies and liver biopsy.

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Tests of Hepatocellular Injury

Increases in either ALT or AST activity result from hepatocellular membrane damage and leakage of the enzymes. This can be due to hepatocyte necrosis (cell death) or degeneration (potentially reversible). Canine and feline hepatocyte cytoplasm is rich in ALT and contains lesser amounts of AST. Altered permeability of the hepatocellular membrane caused by direct injury or a metabolic disturbance releases enzymes into the serum,[2] which is why ALT and AST are thought of as hepatocellular "leakage" enzymes. The magnitude of increase crudely reflects the number of affected hepatocytes. Alanine aminotransferase and AST are neither specific for the etiology of liver disease nor predictive of the outcome. The plasma half-life of ALT activity is about 2.5 days in dogs and much shorter in cats.[2] Thus, persistent increases in ALT over time reflect continued hepatocyte injury. ALT increases should be investigated when they are greater than twice the normal reference interval or persistently abnormal over several months.[3] Following an acute reversible injury, it may take several weeks for ALT to normalize.[4]

A variety of tissues, notably skeletal muscle, liver and red blood cells, contain high AST activity. Aspartate aminotransferase elevations are more sensitive, but less specific, for liver disease than ALT.[5] Hepatic AST is found in hepatocyte cytosol but is predominantly concentrated in the mitochondria (~80%). Marked increases in hepatic AST concentrations generally indicate more severe hepatic damage resulting from hepatic mitochondrial AST release. AST elevations parallel ALT elevations, but at a much lower magnitude. AST elevations that are significantly higher than ALT concentrations suggest skeletal muscle damage and should be further clarified as muscle-tissue origin by the measurement of the serum creatine kinase (CK) activity.[6] The half-life for AST activity in dogs is shorter (< 1 day) than ALT.[2] Following an acute liver injury and during recovery, AST concentrations normalize more rapidly than ALT due to these half-life differences.

In cats, both ALT and AST tend to be less indicative of primary hepatic disease, as most feline liver diseases are cholestatic in nature. The half-life of both enzymes is quite short and may explain the variability of ALT and AST values observed in various types of liver disease of cats.[7]

Tests of Cholestasis or Enzyme Induction

Both ALP and GGT are membrane-bound enzymes that generally reflect hepatic cholestasis; defined as decreased flow of bile.[6] Alkaline phosphatase tends to be associated with the canalicular membranes and GGT with the bile ductular epithelium. Both show minimal activity in normal hepatic tissue but can become elevated due to increased enzyme production being stimulated by either impaired bile flow or drug induction. With cholestasis, surface tension in the canaliculi and bile ductules increases, these surface enzymes are then upregulated into production, solubilized and released into the blood.

Sensitivity	refers to the test's ability to correctly detect patients with liver disease.
Specificity	relates to the test's ability to correctly identify patients without liver disease.

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In dogs, ALP has a high sensitivity (80%), but a low specificity (51%), for hepatobiliary disease.[8] The low specificity is because total serum ALP comprises three major isoenzymes; liver, bone and corticosteroid. Bone ALP is increased with osteoblastic activity (young growing dogs), osteomyelitis, osteosarcoma and other neoplasias. Corticosteroid ALP is also located on the canicular membrane and is induced by exogenous or endogenous corticosteroids. The half-life of liver ALP in dogs is 70 hours, while in cats it is 6 hours.[4,8] In cats, because ALP has a short half-life, is not induced by corticosteroids and has lower total hepatic concentrations, its elevation does not reach the magnitude seen in dogs with a similar disease state. Feline ALP is less sensitive (50%), but a high specificity (93%), for hepatobiliary disease than canine ALP.[8]

The most common cause of abnormal liver enzymes is not primary liver disease, but rather the result of reactive hepatic changes occurring secondary to other, non-hepatic diseases.

Hepatic GGT is located predominantly on the canalicular membranes and bile duct epithelium. Chronic elevations in GGT tend to reflect hepatobiliary tract involvement. In dogs, GGT has a lower sensitivity (50%), but higher specificity (87%), for hepatobiliary disease than total ALP.[2] If an elevated ALP is present with a concurrent increase in serum GGT, specificity for liver disease increases to 94%.[4] The most marked elevations in GGT result from diseases of the biliary epithelium. In cats, GGT has a higher sensitivity (86%), but lower specificity (67%), for hepatobiliary disease than ALP.[8] The most common causes of both elevations in GGT and ALP in one large case series of cats included bile duct obstruction, cholangitis/cholangiohepatitis and cirrhosis.[8] In cats with hepatic lipidosis, serum ALP is usually quite high and GGT is generally normal or only mildly elevated.[8]

Serum ALP and GGT activity can be induced by glucocorticoids (endogenous, topical or systemic), anticonvulsant medications (phenobarbital) and possibly other drugs or herbal supplements in dogs. There is remarkable individual variation in the magnitude of these increases without concomitant hyperbilirubinemia. A moderate to marked increase in serum ALP activity without concurrent hyperbilirubinemia is most compatible with drug-induction and warrants a review of the patient's history (topical or systemic glucocorticoids) or evaluation of adrenal function. The increased ALP has long been attributed to a glucocorticoid-stimulated production (hepatic gene induction) of a novel ALP isoenzyme in the dog and can be distinguished from the cholestasis-induced hepatic ALP isoenzyme by several laboratory procedures.[9] It was initially thought that the glucocorticoid-associated isoenzyme could be used as a marker of exogenously administered corticosteroids or increased production of endogenous glucocorticoids. Unfortunately, the glucocorticoid-associated isoenzyme is also associated with hepatobiliary disease and differentiation of steroid-associated from liver-associated ALP elevations is rarely helpful.[9] The type and duration of systemic corticosteroid use in the dog results in variable ALP responses, and it may take 3 to 6 weeks for serum ALP to return to normal following discontinuation of corticosteroid administration.[10] Cats lack glucocorticoid ALP induction or development of a steroid hepatopathy, therefore increases in ALP do not result for these reasons.

Tests Evaluating Liver Function: Liver Products

The evaluation of liver function depends on tests that reflect alterations in the synthetic or excretory capacity of the liver. Synthetic failure is generally observed when greater than 60%-70% of the liver loses function because of the great hepatic reserve capacity.[3] There is, however, no specific liver function test that reflects the overall functional status of the liver. On a routine biochemical profile, it is important to understand that abnormal liver function could affect bilirubin, albumin, glucose, cholesterol and/or BUN.

Bilirubin is the most sensitive and specific function test of hepatobiliary tract disease once hemolytic disease has been ruled out. Bilirubin is affected by hepatocellular metabolism (uptake, protein binding, conjugation, and excretion) and alteration in biliary excretion (extrahepatic biliary obstruction). Metabolic conditions such as endotoxins, inflammatory cytokines, fatty acids, and protein deficiency can interfere with bilirubin metabolism without structural damage to the hepatocyte or bile ducts. This is often referred to as cholestasis of sepsis and frequently hepatic enzymes are often normal.[11] Cats tend to develop increases in bilirubin concentrations quite easily that are not associated with primary liver disease, but due to factors altering hepatic bilirubin metabolism.[12]

Albumin is exclusively made in the liver and, if not lost from the body (gastrointestinal or renal), sequestered or diluted, a low concentration would suggest significant hepatic dysfunction. It may take greater than 60%-70% hepatic dysfunction for albumin concentrations to decline.[13] Albumin is also a negative acute phase reactive protein, so inflammatory conditions could also lower albumin concentrations due to transiently reduced production.[14] Hypoglycemia occurs when 75% or greater liver mass is lost.[15] Cholesterol is quite variable and is often low in dogs having portosystemic shunts (PSS) and is elevated in cholestatic or obstructive liver disease. The BUN may be low in dogs having a PSS or chronic liver disease from the failure to convert ammonia to BUN. Other causes of a low BUN include low dietary protein intake or starvation. Major clotting factors (except Factor VIII) and fibrinogen are made in the liver, therefore prolonged clotting time suggests significant hepatic dysfunction or factor consumption. Low fibrinogen concentrations with liver disease also suggest reduced hepatic function.[16]

Blood Ammonia

Blood ammonia or the ammonia tolerance test are infrequently performed but tend to reflect abnormal hepatic portal shunting (acquired or congenital shunts) or to detect significant hepatocellular dysfunction (likely greater than 70% hepatic dysfunction).[17] The liver detoxifies ammonia, primarily arising from the gastrointestinal tract, then converts ammonia to urea. Elevated fasting blood ammonia levels (>46 µmol/L) have been shown to be a sensitive (98%) and specific (89%) test for the detection of congenital or acquired PSS in dogs.[18] Because of the complexities in sample collection and performing ammonia tests, it is not routinely used in clinical situations. Dry chemistry tests for blood ammonia provide variable results.[19]

Bile Acids

Serum bile acids (BA) are thought to be the most sensitive liver function test that is readily available for use in small animals. Bile acids are used to screen patients with abnormal liver enzymes to determine if there could be loss of hepatic function and for patients suspected to have PSS (either congenital or acquired). Bile acids are synthesized from cholesterol in the liver, then conjugated and excreted into the bile. They are then transported to the gallbladder (following a meal), released into the intestine (to emulsify fat), actively resorbed, return to the liver, and then re-circulated back into the bile. Only a small fraction of the total BA pool ever escapes into the systemic circulation. Thus, the enterohepatic circulation of BA occurs with an approximate 95%-98% efficiency.[20]

Standard BA testing involves collection of both a pre- and post-prandial blood sample. Performing pre- and post-prandial sample testing increases the sensitivity and specificity of the test.[6] The pre-prandial sample is collected after a 12-hour fast. Normal pre-prandial or fasting serum bile acid concentration (FSBA) reflects an efficient and intact enterohepatic circulation. Pathology of the hepatobiliary system or the portal circulation results in an increased FSBA prior to the development of hyperbilirubinemia, negating its usefulness in the icteric patient. A FSBA increase is not specific for a particular type of pathologic process but is associated with a variety of hepatic insults or abnormalities of the portal circulation.

The diagnostic value of determining post-prandial BA (PPBA) concentration is increased sensitivity for the detection of hepatic disease and congenital, portal vascular anomalies. In dogs, the specificity of fasting and postprandial bile acids for hepatobiliary disease are both high, when cutoff values are used. In one study specificity of FSBA and PPBA was 95% and 100%, respectively.[21] When using these guidelines, it is prudent to recognize that a small number of healthy dogs have been reported with PPBA values above the cutoff. [Always refer to one's particular laboratory for normal bile acid reference intervals in making clinical decisions.] The determination of total bile acids can contribute to the decision to obtain histological support for the diagnosis of hepatic disease.

The Approach to Abnormal Liver Enzymes

Generally, unexplained, elevated liver enzymes in the non-clinical patient persisting over 6-8 weeks become an indication to further evaluate the liver.

A general algorithm for the work-up of dogs having abnormal liver enzymes (ALT, AST, ALP and or GGT) can be useful in interpretation and diagnosis. [Refer to Figure 1.] Abnormal liver enzymes are usually detected either during the evaluation of the sick patient or during routine wellness or preanesthetic screenings. The most common cause of abnormal liver enzymes is not primary liver disease, but rather the result of reactive hepatic changes occurring secondary to other, non-hepatic diseases.[6] The non-clinical patient with an increased liver biochemical test result should have the value(s) confirmed at least once to exclude a spurious result from laboratory error and to avoid unnecessary and costly additional testing. A careful history is essential to exclude drug-associated enzyme elevations. That history should also document all non-traditional therapies, such as herbal supplements or nutraceuticals, as they could potentially cause a drug-induced hepatopathy.

Next, the signalment of the patient may also provide an insight to a possible etiology. Old dogs tend to get more diseases than the young patient. For example, old dogs frequently have benign hepatic nodular hyperplasia, endocrine disease, many types of neoplasia, or systemic disease. The most common primary hepatic disease in dogs is chronic hepatitis. Chronic hepatitis is observed in younger to middle-aged dogs with certain breeds being predisposed. Dogs with early chronic hepatitis do not usually display clinical signs and have only abnormal liver enzyme activities on a biochemical panel. As the disease progresses, clinical signs of liver disease are likely to occur.

Table 1

Some Common Rule-outs for Hepatobiliary Disease Causing Hepatic Enzyme Elevations[3]
Idiopathic inflammatory hepatopathy (chronic hepatitis, cholangitis)
Copper-associated hepatopathy
Infectious hepatopathy (bacterial, leptospiral, viral)
Biliary tract disorders (cholelithiasis, mucocele)
Hepatic vacuolar disorders
Congenital ductal plate anomaly
Benign hepatic nodular hyperplasia
Hepatic neoplasia

Examples of non-hepatic conditions would include intra-abdominal disorders (inflammatory bowel disease (IBD), pancreatitis, nutritional abnormalities), cardiovascular disease, or metabolic derangements (hypothyroidism, Cushing's disease). Generally, these secondary hepatic changes are reversible once the primary disease is treated or managed. Successful resolution of the non-hepatic disease with continued abnormal liver enzymes is a strong indication for further investigation of liver function.

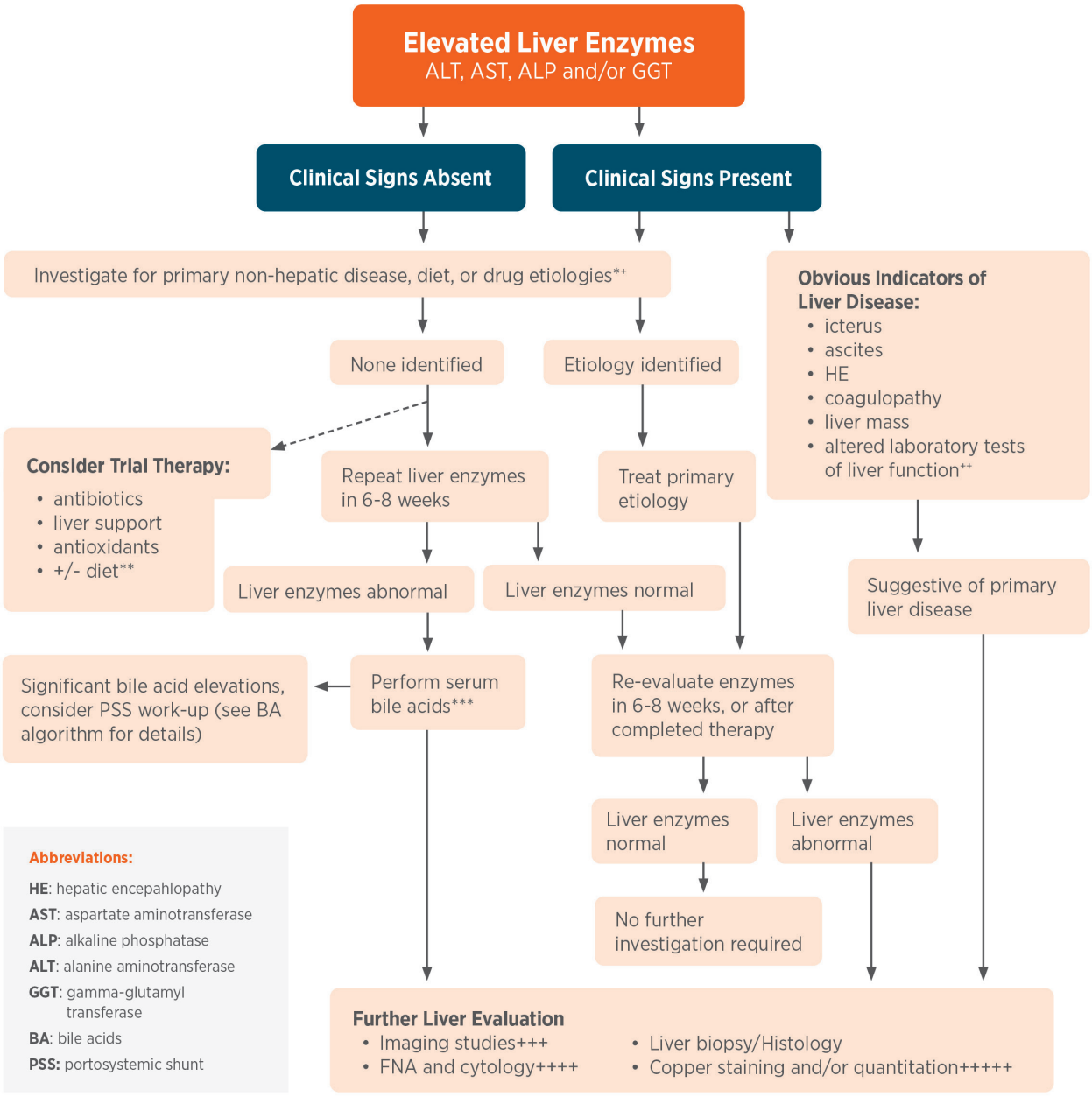
Table 2

Some Common Rule-outs for Non-Hepatic Disorders Causing Hepatic Enzyme Elevations[3]
Endocrine disorders (diabetes mellitus, hypo and hyperthyroidism, hyperadrenocorticism)
Gastrointestinal disorders (IBD, pancreatitis)
Metastatic neoplasia
Drug or toxin-induced hepatopathy (including supplements)
Nutritional abnormalities
Cardiovascular disease (hypoxia, hypotension)
Sepsis or infection

If there is no evidence of a primary non-hepatic disorder or a drug history to explain abnormal liver enzymes, then the liver should be further assessed. Evaluation of the liver may include liver function tests, coagulation profiles, and imaging studies. Generally, the next step in liver evaluation involves imaging

using radiographs or preferably liver ultrasound. Ultrasound examination of the liver is useful for detecting hepatic masses, parenchymal changes and the extra-hepatic biliary system. Ultrasound however does not provide a histological diagnosis.[22] During ultrasound, fine needle aspiration of the liver with cytology are routinely performed. It is important to note that hepatic cytology poorly correlates with a histopathology interpretation, with the best correlation being neoplasia and diffuse vacuolar disease.[23] Imaging, biochemical testing, and liver cytology cannot replace a liver biopsy. A liver biopsy is required for a definitive determination of the nature and extent of hepatic damage and to appropriately direct the course of treatment. The method for liver biopsy procurement may be surgical, laparoscopic, or needle (14-16 g) biopsy.[24] Each has inherent advantages and disadvantages and the decision on which procedure to use should be made in light of all other diagnostic information, and always considering what is in the best interest of the patient and the client.

Figure 1. Abnormal Liver Enzyme Work-Up Algorithm



* Consider endocrine disease, dental disease, chronic pancreatitis and GI disease. When investigating drugs include herbals and nutraceuticals. Altered liver function found on a common chemistry panel may include: unexplained low glucose, albumin, BUN, and/or cholesterol.
** Trial antibiotic therapy may be indicated if a possibility of infection. Hypoallergenic diet trials if a possibility of GI disease. Antioxidant therapy may improve oxidative status of the liver. Also consider treatment trials if a comprehensive liver work-up is unlikely to be owner-approved.
*** Identifying both abnormal bile acids and liver enzymes are a strong indication for a complete liver evaluation. Bile acids >100µM/L with normal bilirubin should suggest possibility of portosystemic shunting (congenital or acquired). Performing a fasted and postprandial sample improves diagnostic sensitivity of the test.
+ Always consider leptospirosis and pancreatitis in the work-up.
++ In some patients the history, signalment and physical exam indicate primary liver disease. Tests that reflect altered liver function include albumin, BUN, glucose, bilirubin, cholesterol and coagulation tests.
+++ Imaging studies include routine abdominal radiographs, ultrasound and CT or CT angiograms.
++++ Fine needle aspiration (FNA) and cytology have poor diagnostic accuracy except for neoplasia and diffuse vacuolar disease.
+++++ Copper evaluation should be considered in all inflammatory liver disorders.

Identification of the non-clinical patient having increased liver enzymes becomes more problematic. As stated above, the history and physical examination should be comprehensive, looking for clues of occult disease. If there is no drug history or clues to other systemic disease, it is rational to simply re-evaluate the patient in approximately 6-8 weeks.[3] If after an appropriate period of observation, the liver enzymes are still abnormal and unexplained, then further liver work-up is indicated. Serum bile acids should be performed and, if abnormal, either indicate PSS or altered liver function and should prompt further hepatic evaluation. Generally, unexplained, elevated liver enzymes in the non-clinical patient persisting over 6-8 weeks become an indication to further evaluate the liver.[3] In most cases, delaying that work-up in a non-clinical patient will not likely result in significant, progressive hepatic deterioration, if the patient remains clinically normal. During the waiting period, hepatic supportive therapy (using various antioxidants such as SAMe, milk thistle products or others) may be rational to provide, especially if an advanced work-up is unlikely to occur. In appropriate circumstances, one may also consider trial therapy using antibiotics as empirical therapy for bacterial cholangitis, chronic leptospirosis or other infectious causes.[25]

5 Key "Take Home" Points

1. Abnormal liver enzymes should not be ignored.
2. ALT and AST increases represent hepatocellular degeneration or necrosis.
3. ALP isoenzymes of significance represent bone, liver, or glucocorticoid-induced in dogs.
4. Abnormal bile acids represent portosystemic shunting or hepatocellular dysfunction.
5. A liver biopsy is required for definitive diagnosis in most cases of primary hepatic disease.

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